Prospective, multicenter, comparative, parallel study to validate a microRNA-based fecal test for colorectal cancer screening. The miRFec study.

NCT Id: not yet assigned

Protocol code: miRFec001

Protocol version and date: version 2 (January 28th, 2022)

Promoter: *Fundació Clínic per a la Recerca Biomèdica* (Clinic Foundation for Biomedical Research)

Coordinator and principal investigator: Dr. Antoni Castells. Gastroenterology Department, Hospital Clínic of Barcelona

1. SUMMARY

1.1 Title:
Prospective, multicenter, comparative, parallel study to validate a microRNA-based fecal test for colorectal cancer screening. The miRFec study.

1.2 Promoter:
Fundació Clínic per a la Recerca Biomédica (Clinic Foundation for Biomedical Research)

1.3 Protocol code:
miRFec001

1.4 Coordinator and principal investigator:
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1.5 Researchers and centers where the study is planned to be performed:
- Hospital Clinic of Barcelona, Spain - Dr. Antoni Castells
- Hospital del Mar, Barcelona, Spain - Dr. Xavier Bessa
- Hospital General de Alicante, Spain - Dr. Rodrigo Jover
- Hospital de Ourense, Spain - Dr. Joaquín Cubiella
- Hospital Universitario de Canarias, Spain - Dr. Goretti Hernández
- Hospital de Donostia, Spain - Dr. Luis Bujanda
- National Research Institute of Oncology, Warsaw, Poland - Dr. Michael Kaminiski and Dr. Joanna Nowakowska
- Endotherapy, Polish Foundation of Gastroenterology, Warsaw, Poland - Dr. Bronisław Kotowski
- Endomed, Gorzow Wielkopolski, Poland - Dr. Marek Buszkiewicz
- Endomed, Radom, Poland - Dr. Janusz Romanowski
- HT Centrum, Tychy, Poland - Dr.Tomasz Romańczyk
- Centrum Gastroenterologii, Wodzislaw Slaski, Poland - Dr. Krzysztof Jarus

1.6 Ethics Committee (CEIm):
Hospital Clínic of Barcelona, Spain

1.7 Population included:
Study population will include 9,670 individuals:
- Male and female aged 50 to 75 (average-risk) referred to colonoscopy-based colorectal cancer (CRC) screening
- Male and female aged 30 to 80 with family history of CRC (moderate-risk) referred to colonoscopy-based CRC screening

1.8 Study design and phase:
Prospective, multicenter, comparative, parallel, *in vitro* diagnostic (IVD) clinical performance study

1.9 Primary endpoint:

To compare the performance (i.e. sensitivity) of the miRFec test with respect to fecal immunochemical testing (FIT) for the detection of advanced colorectal neoplasm (i.e. CRC, advanced adenomas [AA] or advanced serrated lesions [ASL]).

1.10 Secondary endpoints:

- To compare the specificity of the miRFec test with respect to FIT
- To compare the sensitivity of the miRFec test with respect to FIT for the detection of CRC
- To compare the sensitivity of the miRFec test with respect to FIT for the detection of AA
- To compare the sensitivity of the miRFec test with respect to FIT for the detection of ASL
- To compare the detection rate for advanced colorectal neoplasm of the miRFec test with respect to FIT
- To compare discrimination capacity of the miRFec score and fecal hemoglobin concentration for the detection of advanced colorectal neoplasm
- To compare the discrimination capacity of the miRFec score and fecal hemoglobin concentration for the detection of CRC
- To compare the cost-effectiveness for advanced colorectal neoplasm detection of the miRFec test with respect to FIT

1.11 Main variable

Sensitivity for the detection of advanced colorectal neoplasm (i.e. CRC, AA or ASL)

1.12 Secondary variables

- Specificity
- Sensitivity for the detection of CRC, AA and ASL
- Detection rate for advanced colorectal neoplasm
- Discrimination capacity for advanced colorectal neoplasm and CRC
- Cost-effectiveness for advanced colorectal neoplasm detection

1.13 Inclusion criteria

- Male or female aged 50 to 75 (average-risk) referred to colonoscopy-based CRC screening
- Male or female aged 30 to 80 with family history of CRC (moderate-risk) referred to colonoscopy-based CRC screening

1.14 Exclusion criteria

- Lack of informed consent to participate
- Personal history of CRC, regardless when it was diagnosed
- Personal history of any other cancer in the last five years, except for non-melanoma skin cancer
- Personal history of Lynch syndrome
- Personal history of adenomatous or hamartomatous polyposis
- Personal history of serrated polyposis syndrome
- Personal history of inflammatory bowel disease
- Personal history of total colectomy for any reason
- Family history of Lynch syndrome
- Family history of adenomatous or hamartomatous polyposis
- Family history of serrated polyposis syndrome

1.15 Follow-up and timeline

- Phase I: threshold search (February 2022 – July 2023)
- Phase II: performance evaluation (August 2023 – July 2025)

2. ABBREVIATIONS

AA	Advanced adenoma
AE	Adverse event
ASL	Advanced serrated lesion
BBPS	Boston bowel preparation scale
CRC	Colorectal cancer
e-CRF	Electronic case report form
FIT	Fecal immunochemical test
miRNA	Micro RNA
IVD	*In vitro* diagnostic
SAE	Serious adverse event
SAP	Statistical analysis plan
SAR	Serious adverse reaction
SPS	Serrated polyposis syndrome

3. INDEX

4. GENERAL INFORMATION

4.1. Study identification

- Title: Prospective, multicenter, comparative, parallel study to validate a microRNA-based fecal test for colorectal cancer screening. The miRFec study.
- Protocol code: miRFec001
- Version 1. Date: June 16th, 2021
- Version 2. Date: January 28th, 2022

4.2. Promoter identification

Fundació Clínic per a la Recerca Biomèdica (Clinic Foundation for Biomedical Research)
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4.3. Coordinator and principal investigator

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5. JUSTIFICATION

Colorectal cancer (CRC) is the third most common cancer and the second leading cause of cancer-related death in Western countries, accounting for almost 1.8 million of new cases and 800.000 deaths in 2018[1]. Although 90% of patients diagnosed at early stages have an overall survival of more than 5 years, this figure decreases to 10% in patients diagnosed at advanced stages with distant metastasis[2]. Interestingly, evidence from several studies has shown that CRC screening is effective and cost-effective in the average-risk population[3].

Recommended CRC screening strategies fall in two broad categories: stool tests (occult blood –guaiac test and fecal immunochemical test [FIT]– or exfoliated DNA tests) and structural exams (flexible sigmoidoscopy, colonoscopy and CT colonography)[4]. While structural exams detect both cancer and premalignant lesions, stool tests primarily identify cancer because of their limited sensitivity to detect advanced adenomas (AA)[5]. Moreover, in a two-step screening scenario, which is the most extended worldwide[2], the relatively low specificity of the initial examination results in a high false-positive rate, leading to a significant number of unnecessary colonoscopies[6].

It is important to point out that to maximize the impact and ensure high coverage and equity of access, population-based organized screening programs are highly recommended, as opposed to case-finding or opportunistic screening, since they have an explicit policy and a team responsible for organization and invitation processes as well as to monitor and improve quality and outcomes[7]. In that context, almost all countries have chosen a two-step screening with FIT as the first step. It is important to note, however, that although the highest level of evidence supports the implementation of FIT-based screening, many countries have constrained their programs to manage the resultant colonoscopy workload in an equitable, timely, and high-quality manner[2]. For instance, countries such as England or Australia have initially constrained the age range of those offered screening, while others such as the Netherlands and Scotland have tailored the sensitivity of FIT –using a higher threshold– to reduce colonoscopy needs[2].

The use of biomarkers for screening purposes appears as an appealing approach to overcome the above-mentioned limitations[8]. MicroRNAs (miRNA) are small endogenous non-coding RNAs of 18 to 25 nucleotides that negatively regulate gene expression at post-transcriptional level by either repressing transcript translation or inducing the degradation of target mRNAs[9]. It is important to highlight that they are involved in several biological processes, i.e. carcinogenesis[10]. Indeed, deregulated miRNAs reflect physiopathological states and allow distinguishing different stages and subtypes of cancer, including CRC, as well as to discriminating neoplastic patients from healthy individuals[11]. Interestingly, it seems that precursor lesions also show an altered miRNA pattern that could be, in part, secreted to the extracellular milieu. As a consequence, miRNAs detected in different body fluids, i.e. feces[12], have been suggested as potential biomarkers for CRC screening. In that sense, in a recent study, we have technically and clinically validated a predictive algorithm based on a fecal miRNA signature –the miRFec test, a Gradient Boosting Machine-generated algorithm that includes two fecal miRNAs (miR-421 and miR-27a-3p) and fecal hemoglobin concentration, along with age and gender– able to identify patients with advanced colorectal neoplasm more accurately than hemoglobin concentration in FIT-positive individuals[13, 14].

The present study aims to compare the effectiveness and cost-effectiveness of the miRFec test concerning FIT for the detection of advanced colorectal neoplasm among individuals participating in CRC screening.

6. PURPOSE OF THE STUDY

6.1. Hypothesis

The miRFec test –a Gradient Boosting Machine-generated algorithm that includes two fecal miRNAs (miR-421 and miR-27a-3p) and fecal hemoglobin concentration, along with age and gender– is more effective and cost-effective than FIT for the identification of patients with advanced colorectal neoplasm (i.e. CRC, AA or advanced serrated lesions [ASL]) among individuals participating in CRC screening programs.

6.2. Primary endpoint

The main goal of the study is to compare the sensitivity of the miRFec test with respect to FIT for the detection of advanced colorectal neoplasm.

6.3. Secondary endpoints

- To compare the specificity of the miRFec test with respect to FIT
- To compare the sensitivity of the miRFec test with respect to FIT for the detection of CRC
- To compare the sensitivity of the miRFec test with respect to FIT for the detection of AA
- To compare the sensitivity of the miRFec test with respect to FIT for the detection of ASL
- To compare the detection rate for advanced colorectal neoplasm of the miRFec test with respect to FIT
- To compare the discrimination capacity of the miRFec score and fecal hemoglobin concentration for the detection of advanced colorectal neoplasm
- To compare the discrimination capacity of the miRFec score and fecal hemoglobin concentration for the detection of CRC
- To compare the cost-effectiveness for advanced colorectal neoplasm detection of the miRFec test with respect to FIT

6.4. Main and secondary variables

- Main variable: Sensitivity for the detection of advanced colorectal neoplasm (i.e. CRC, AA or ASL)
- Secondary variables: specificity; sensitivity for the detection of CRC, AA and ASL; detection rate for advanced colorectal neoplasm; discrimination capacity for advanced colorectal neoplasm and CRC; cost-effectiveness for advanced colorectal neoplasm detection

7. STUDY DESIGN

Prospective, multicenter, comparative, parallel, *in vitro* diagnostic (IVD) clinical performance study, carried out in medical centers of Spain and Poland.

Eligible individuals will undergo two different examinations –miRFec test and FIT– in a unique stool sample collected at home. Results of both tests will be compared to those obtained in the colonoscopy (with pathology results when colorectal lesions were identified), which will be used as the reference method.

7.1. Study population

Study population will include 9,670 individuals:

- Male and female aged 50 to 75 (average-risk) referred to colonoscopy-based colorectal cancer (CRC) screening.
- Male and female aged 30 to 80 with family history of CRC (moderate-risk) referred to colonoscopy-based CRC screening.

7.2. Study duration

The study will last up to 5 years, which includes two consecutive stages: phase I (threshold search), aimed at establishing the most adequate threshold of miRFec test for advanced colorectal neoplasm detection, and phase II (performance evaluation), to compare the clinical performance of the miRFec test with respect to FIT.

7.3. End of study

The recruitment process of the study will end up when completing the required sample size (9,670 individuals). On the other hand, the study will finish when results obtained in both the miRFec test and the FIT are correlated with colonoscopy (and pathology) findings.

8. PARTICIPANTS

The study population will include individuals undergoing colonoscopy as primary screening approach in the context of both average and moderate-risk screening programs.

8.1. Inclusion criteria

- Male and female aged 50 to 75 years (average-risk) referred to colonoscopy-based CRC screening. This group includes individuals without family history of CRC as well as those with a family history limited to one first-degree relative with CRC diagnosed after the age of 50 or any number of second- and third-degree relatives with CRC regardless the age of diagnosis.
- Male or female aged 30 to 80 years with family history of CRC (moderate-risk) referred to colonoscopy-based CRC screening. More specifically, this condition corresponds to individuals with two or more first-degree relatives with CRC regardless the age of diagnosis, or one first-degree relative with CRC diagnosed before the age of 50.

8.2. Exclusion criteria

- Lack of informed consent to participate
- Personal history of CRC, regardless when it was diagnosed
- Personal history of any other cancer in the last five years, except for non-melanoma skin cancer
- Personal history of Lynch syndrome
- Personal history of adenomatous or hamartomatous polyposis
- Personal history of serrated polyposis syndrome
- Personal history of inflammatory bowel disease
- Personal history of total colectomy for any reason
- Family history of Lynch syndrome
- Family history of adenomatous or hamartomatous polyposis
- Family history of serrated polyposis syndrome

8.3. Withdrawal criteria

According to Helsinki's Declaration, all subjects participating in the study can withdraw whenever, without giving any reason and with no repercussion in their medical care. Any withdrawal from the study must be informed to the monitor of the study and documented in the e-CRF and participant's medical record. Withdrawal criteria includes:

- Exclusion criteria not reported at baseline visit
- Participant does not deliver the fecal sample
- Poor fecal sample quality (overfilling or insufficient amount)
- Participant does not undergo colonoscopy
- Inadequate bowel preparation in three consecutive colonoscopies.

9. PROCEDURES

9.1. Recruitment

All individuals included in average-risk and moderate-risk CRC screening programs carried out in participating centers are scheduled for a medical or nurse visit, in which they are asked for demographic data, personal and family history of cancer, comorbidities, usual medication, as well as any symptom of CRC. Once anamnesis is completed, a screening colonoscopy is scheduled. At this time, individuals will be offered to participate in the present study.

All candidates to participate in the study will be explained orally about purpose of the study and given a written information sheet (Annex 1). Any doubt about the nature of study, benefits and potential risks will be solved. If the subject agrees to participate, informed consent will be signed (Annex 2). It will be informed about the freedom to revoke the informed consent without penalty or changes in clinical management, as well as the protection of their personal data and medical history according to current legislation.

The recruitment will be competitive between all sites.

9.2. Fecal sample collection

Once the subject accepts to participate in the study, a fecal sample collection kit will be delivered together with the sample collection instructions (Annex 3). The sample must be collected at home before starting bowel preparation, more specifically between 24-72 hours before the day that colonoscopy is scheduled.

Sample must be kept in the refrigerator (+4ºC) until the day of colonoscopy, when it must be brought and delivered to the endoscopy unit staff.

9.3. Endoscopy and pathology reporting

All colonoscopies will be performed by experienced endoscopists. Quality of colonoscopy will be ensured following guidelines of the European Society of Gastrointestinal Endoscopy (ESGE)[15] and monitored.

Colon cleansing will be scored according to the Boston bowel preparation scale (BBPS) (Table 1)[16]. In brief, right (i.e. cecum and ascending colon), transverse (i.e. hepatic flexure, transverse colon and splenic flexure) and left (i.e. descending colon, sigmoid and rectum) colon are scored from 0 to 3. These segments' score are summed up for a total BBPS score. A punctuation of minimum 2 is required in each segment of the colon. Not achieving an adequate bowel preparation in three consecutive colonoscopies will be considered a withdrawal criterion of the study.

Table 1. Boston bowel preparation scale

Score	Description
0	Unprepared colon segment with mucosa not seen because of solid stool that cannot be cleared
1	Portion of mucosa of the colon segment seen, but other areas of the colon segment are not well seen because of staining, residual stool and/or opaque liquid
2	Minor account of residual staining, small fragments of stool and/or opaque liquid, but mucosa of colon segment is seen well

Score	Description
3	Entire mucosa of colon segment seen well with no residual staining, small fragments of stool or opaque liquid

All detected lesions must be described, documented and recorded in the electronic case report form (e-CRF). Decision of removing and/or taking biopsies of any detected lesion will correspond to the endoscopist. Similarly, other therapeutic decisions, including need for surgical resection, will correspond to the patient's responsible physician.

Invasive cancer will be considered when malignant cells are observed beyond the *muscularis mucosa*, and graded according to the American Joint Committee on Cancer guidelines (8th version) (Annex 4)[17]. Carcinoma *in situ* (Tis, stage 0) will be considered equivalent to high-grade dysplasia and, therefore, this lesion will be classified as AA.

9.4. Sample processing

Both miRNA expression and FIT will be analyzed in a central laboratory (Hospital Clinic of Barcelona). For this purpose, samples will be kept in a refrigerator (+4ºC) of the participating center until shipping to the central laboratory.

9.5. Definitions

9.5.1. Adenomatous polyposis

- More than 100 adenomatous polyps (classical form) or 20-100 adenomatous polyps (attenuated form) in one or more colonoscopies (cumulative figure), or
- Any number of adenomas in a gene mutation carrier in any causative gene (*APC, MUTYH, POLE, POLD1, NTHL1*)

9.5.2. Hamartomatous polyposis

Any number of hamartomatous polyps in a gene mutation carrier in any causative gene (*SMAD4, BMPR1A, ENG, STK11, PTEN*)

9.5.3. Serrated polyposis syndrome

According to World Health Organization (2019), serrated polyposis syndrome is defined as:

- ≥5 serrated polyps proximal to the rectum, all being at least 5 mm in size, with ≥2 of them >10mm.
- >20 serrated polyps of any size along the colon with ≥ 5 proximal to the rectum

9.5.4. Lynch syndrome

Gene mutation carrier in any DNA mismatch repair gene (MLH1, PMS2, MSH2, MSH6)

9.5.5. Invasive colorectal cancer

Invasive CRC will be considered when malignant cells are observed beyond the *muscularis mucosa*.

9.5.6. Advanced adenoma

Adenomas ≥10 mm in size, or with villous architecture, or high-grade dysplasia or intramucosal carcinoma (Tis) will be classified as AA.

9.5.7. Advanced serrated lesion

Serrated lesions ≥10 mm in size or with dysplasia will be classified as ASL.

9.5.8. Advanced colorectal neoplasm

Advanced colorectal neoplasm will be defined as CRC, AA or ASL.

10. STUDY CALENDAR

Tasks	Baseline visit	Day -3 to -1	Day 0 (colonoscopy)	Day 1 to 7	Case completion
Inclusion/exclusion criteria check	✓				
Participant information sheet delivery	✓				
Consent form signature	✓				
Kit and fecal sample collection instructions delivery	✓				
At home fecal sample collection		✓			
Kit (fecal sample) return			✓		
Colonoscopy			✓		
Shipping samples to central laboratory				✓	
Withdrawal criteria check					✓
Introduction of colonoscopy/pathology data into e-CRF					✓

- **Baseline visit.** Study investigators will ask for personal and family history, and check whether candidate fulfill inclusion/exclusion criteria to participate in the study. If that is the case, the subject will get a copy of the information sheet, will sign the consent form, and will be given a kit for fecal sample collection and instructions to collect the sample.
- **Day -3 to -1 (with respect the day of colonoscopy).** The subject will collect a fecal sample at home, 24-72 hours before colonoscopy and always before starting laxatives (bowel preparation). Once the sample is collected, it must be kept in a refrigerator (+4ºC) until transporting it to the endoscopy unit.
- **Day 0 (colonoscopy)**. Patients will deliver the fecal sample to study investigators. Samples must be kept in a refrigerator (+4ºC) of the participating center until shipping to the central laboratory.
- **Day 1 to 7 (with respect to the day of colonoscopy).** Samples will be shipped to the central laboratory once a week, ideally on Monday from Polish centers and Wednesday from Spanish centers.

- **Case completion.** When all information is available, data from colonoscopies, surgical procedures and pathology (if lesions are detected) will be introduced in the e-CRF.

11. SAFETY ASSESSMENT

The investigator is responsible for detecting and documenting any adverse event that fills in with the criteria and definition of severe adverse event that appear in this protocol.

11.1. Safety parameters definitions

- **Adverse event (AE).** Any harmful incident for a patient participating in a clinical trial using medicines, devices or medical procedures, even though there is no relation between the incident and the treatment used.
- **Serious adverse event (SAE) / Serious adverse reaction (SAR)**. An adverse event or adverse reaction is considered serious if it may cause death, require hospitalization or prolong the duration of it, cause invalidity or permanent or important disability, or cause a congenital malformation. Any suspicion of adverse event considered important in terms of medical point of view, will also be considered as serious and must be reported, even though they do not meet the criteria mentioned above. Additionally, medical staff must notify any suspicion of infectious agent transmission. "Life threat" is understood as a situation in which, in the opinion of physician, the patient was at a real risk of death at the time of the adverse event or the adverse reaction and, had there not been a timely therapeutic intervention, death would have occurred. It does not mean that the adverse event or adverse reaction hypothetically could have caused death if it had been more intense. The concept "require hospitalization" will exclude both planned hospitalizations for scheduled treatments and those that have been planned or anticipated before the start of study concerning a pre-existing medical situation.
- **Unexpected adverse reaction**. It refers to an event or reaction that is not listed in the investigator's brochure or is not listed at the specificity or severity that has been observed.

11.2. Safety parameters evaluation

Participation in this study will not represent any increased risk in addition to those associated with the colonoscopy already indicated for a screening purpose.

For safety evaluation, SAE occurring up to 30 days after colonoscopy will be recorded in the e-CRF. The most frequent SAE include adverse reaction to sedation, bleeding and perforation if any lesion should be resected.

11.3. Register of adverse events

SAE and any defect of the device (i.e. fecal sample collection kit) will be recorded in the e-CRF.

12. STATISTICS

12.1. Sample size calculation

This study includes two consecutive phases. The first one would consist of determining the optimal diagnostic threshold of the miRFec score in a population-based screening setting among *naive* participants. The second stage aims at measuring the comparative performance of the miRFec test with respect to FIT. Therefore, the sample size of the second phase depends upon estimates of the first phase and population characteristics.

In order to validate the hypothesis of the miRFec test having greater performance than FIT, sample size has been calculated to detect the estimated difference using a normal approximation, with a type I error of 0.025 (MH correction), a prevalence of CRC of 5‰, and type II error of 0.1. First simulations suggest a subject population of 3,600 for phase I, and 6,070 for phase II (total, 9,670 individuals). However, sample size requirements may vary depending on advanced colorectal neoplasm prevalence and, consequently, sample size will be recalculated after phase I completion.

12.2. Statistical analysis plan (SAP)

Sensitivity [proportion of persons with disease who have a positive test] of the miRFec test will be compared with the corresponding figure obtained with FIT (cut-off level, 20 µg Hb/g of feces) for the detection of advanced colorectal neoplasm.

Specificity [proportion of persons without disease who have a negative test] of the miRFec test will be compared with the corresponding figure obtained with FIT, with advanced colorectal neoplasms excluded and only non-advanced adenomas and negative results included (primary measure) and with only negative results included (secondary measure).

Discrimination capacity of the miRFec score and fecal hemoglobin concentration will be measured by the area under the receiver operating characteristic (ROC) curve for the detection of both advanced colorectal neoplasm and CRC.

Other parameters to be calculated include positive predictive value [proportion of persons with disease among those with a positive test], negative predictive value [proportion of persons without disease among those with a negative test], and number needed to screen [number of persons who would need to be screened to identify one person with the disease] using either miRFec test or FIT.

For test characteristics, 95% lower boundaries will be computed with the use of an exact binomial test. Lower 95% confidence limits for comparative analyses were computed with the use of a one-sided McNemar paired-comparisons test for the observed difference in sensitivity between the miRFec test and FIT. The Hanley-McNeil method will be used to calculate p values for the analysis of the ROC curve.

No interim analysis of the data is planned.

13. ETHICS AND LEGAL ASPECTS

The study will be carried out according to current legislation *(*14/2007 of July 3rd on Biomedical Research*)* and Helsinki Declaration guidelines approved by the 18th World Medical Association in Helsinki, Finland, in 1964, and amended in subsequent assemblies in Tokyo 1975, Venice 1983, Hong King 1989, Somerset West 1996, Edinburgh 2000, Seoul 2008, and Fortaleza 2013.

The current protocol has been elaborated according to the EU 2016/679 Regulation of European Parliament related to the protection of natural persons in terms of personal data treatment and free circulation of these data and ICH E6 (R2) Good Clinical Practice.

The study has been submitted to the Research Ethics Committee (CEIm) of Hospital Clinic of Barcelona, and it will be submitted to the corresponding body of other participating centers if needed.

All individuals will provide written informed consent to participate in the study.

14. DATA MANAGEMENT, CONFIDENTIALITY AND REGISTER FILE

14.1. Confidentiality

Treatment, communication and personal data transfer of all participants will comply with EU Regulation 2016/679 of the European Parliament and of the Council of April 27th, 2016 on the protection of natural persons with regard to the processing of personal data and on the free movement of such data, and repealing Directive 95/46/EC (General Data Regulation), being mandatory since May 25th, 2018. The legal basis that justifies the processing of personal data is the consent given in this act, following the provisions of Article 9 of EU Regulation 2016/679.

Data will be collected and identified by a code, so no information to identify the participants will be included. Only study investigator and his/her collaborators with specific permission will be allowed to relate the data collected in the study with participant's medical record.

Identity of participants will not be available to any other person except for a medical emergency or legal requirement.

Health authorities, the Research Ethics Committee and authorized personnel by the study promoter may have access to identified personal information when necessary, to verify data and study procedures, but always maintaining confidentiality according to the current legislation.

Only coded data will be transferred to third parties and other countries, which in no case will contain information that can directly identify the participant (such as name and surname, initials, address, social security number, etc.). If this transfer occurred, it would be only for the study and guaranteeing confidentiality.

If a transfer of encrypted data is made outside the EU, either to entities related to the hospital center of participant, to service providers or researchers who collaborate with participant's physician, all data will be protected by safeguards such as contracts or other established mechanisms established by data protection authorities.

Study promoter promises to process the data following EU Regulation 2016/679 and, therefore, to keep a record of treatment activities that are carried out and to perform a risk assessment treatment to know what measures should be applied and how to do it.

In addition to the rights that the previous legislation already contemplated (access, modification, opposition and cancellation of data, deletion in the new Regulation), any participant can also limit the processing of incorrect data, request a copy or that the data provided for the study can be transferred to a third party (portability). To exercise these rights, participant should contact Principal Investigator of the study or the Data Protection Officer of the Hospital Clinic of Barcelona (protecciodades@clinic.cat). Moreover, participant has the right to contact the Data Protection Agency if he/she is not satisfied.

Data already collected cannot be removed even if participant leave the study, to ensure the validity of the research and to come into effect with legal duties and drug authorization requirements. But no new data will be collected if he/she decides to stop participating.

It is obligatory for researchers and study promoter to keep the data collected for the study at least 25 years after its completion. Subsequently, personal information will only be kept by the center for participant health care, and by the promoter for other scientific research purposes if the participant has given its consent to do so and if this is allowed by the law and applicable ethical requirements.

14.2. Electronic case report form (e-CRF)

All study data will be recorded in the e-CRF, which will be developed by Persei Vivarium using ReseaArch®, an electronic data capture solution that allows data collection in a simple way through forms that validate the information entered, ensuring its quality and reliability. It's built over a safe and trusted eHealth architecture with 21 CFR Part 11 compliance.

Data managed on this project will be anonymous. The forms will not collect data that allows a case identification.

Main core features of ReseaArch® includes:

- e-CRF. Allows the entry of cases. When the data is entered, the information is checked and validated.
- Descriptive statistics. This module provides histograms, pie and bar charts, and statistics like average, deviation, etc.
- Filters. Allows the analysis of subgroup of data and variables fulfilling specific or combined criteria.
- Export data, in an excel file and manage the information by yourself.
- Follow-up module. Includes two online reports: upcoming and overdue follow-ups.
- Patients per hospital. Report that shows the number of patients included by each hospital.
- Global view report. Online report showing the global project status.
- Pending data. List of participants for whom the introduction of some information is still pending.

Information recorded in the e-CRF will be structured in 4 blocks:

- Enrollment, eligibility, demography, and kit delivery
- Fecal sample collection
- Study withdrawal, colorectal findings and colonoscopy-related adverse events
- Laboratory results

Enrollment: country and participating center. Once this information recorded, a unique ID number will be automatically assigned to each patient. For anonymity reasons, name and surname, and medical record number of participants will be kept in a independent, local registry, not available for the coordinating center.

Eligibility: includes fulfillment of inclusion and exclusion criteria.

Demography: includes age, gender, ethnicity, number of first-degree relatives with CRC, number of first-degree relatives diagnosed with CRC before the age of 50, and indication for screening colonoscopy (i.e. average-risk or moderate-risk).

Consent form and kit delivery: includes date of inclusion (which correspond to date of consent form signature) and code of fecal sample collecting kit (code bar of the kit will be scanned).

Sample collection: date of fecal sample collection.

Study withdrawal: if a participant is withdrawn from the study, the reason to do so will be recorded.

Colorectal findings: includes number of diagnostic and therapeutic procedures performed, date of each procedure (either colonoscopy and/or surgery), cecal intubation,

BBPS, number of polyps detected, number of polyps resected, number of invasive CRC detected, and number of invasive CRC resected. Pathology information from all detected lesions will also be recorded.

Serious adverse events: related to screening colonoscopy (i.e. perforation, bleeding, or sedative-related adverse event) will be recorded.

Laboratory: fecal hemoglobin concentration, fecal miR-421 expression, and fecal miR-27a-3p expression.

14.3. Data transfer

Data introduced in the e-CRF must be consistent and according to the source documents (medical record, colonoscopy and pathology reports, among others). Only authorized personnel will introduce all data after receiving specific training on the use of e-CRF.

Any change in the initial recorded data will be justified and traceable so that the data is as clean and accurate as possible for analysis. Data quality is improved through a series of scheduled checks that automatically detect out-of-range or abnormal data.

Data entry should not be delayed because of obtaining data source, trying to be as simultaneous as possible to avoid data loss.

The e-CRF has been designed to require only the essential data for the study.

14.4. Source documents

A source document is understood as all observations or annotations recorded about clinical interventions, as well as all reports that are essential for the research study evaluation. Accordingly, source documents for this study include, but are not limited to, medical record, colonoscopy, pathology, and other medical reports.

Whenever possible, the source document must be the original one; however, a clear, legible and exact copy can be accepted. All centers must keep these documents for auditoria or data monitoring.

14.5. Responsibilities

Only authorized and trained personnel will have access to the e-CRF.

Data managers will guarantee access to the system for all authorized persons.

The promoter is responsible for the consistency of data introduced in the e-CRF against the source document.

The promoter is responsible for verification of information on the e-CRF and source document.

15. BIOLOGICAL SAMPLES MANAGEMENT

Participation in this study involves obtaining a stool sample.

Participants agree that samples will be obtained according to biomedical research Law 14/2007 and Royal Decree 1716/2011, which regulate the use of biological samples in research.

Samples will be stored in the laboratory of *Fundació Clínic per a la Recerca Biomèdica* in Barcelona until they are used for this study. Once the study is finished, leftover samples will be destroyed unless participant expresses his/her consent (Annex 2) to store them at the Biobanc of Hospital Clínic-IDIBAPS at the registered collection number C. 0004006, and use them in future research.

A code to identify fecal sample will be used. Only the study's Principal Investigator and his collaborators will be able to associate the sample with the participant. The information obtained from using these samples will be treated in the same way as the rest of the data obtained during this study.

Biological samples transfer for this study is free and voluntary. That means that participant will have no rights to any possible commercial benefit from the findings that could result from biomedical research.

Any relevant information that could affect participant health or his/her family members will be notified. If necessary, investigators will use the data on participant medical record to be in touch with him/her. However, participant willingness to receive no information will be respected.

Additionally, the results obtained if genetic analyses are performed will not be communicated to participant or his/her physician, although he/she can request them by contacting the study's Principal Investigator.

16. FUNDING

Eiken Chemical Co., Ltd, Tokyo, has donated the amount of 30,000,000 JPY (231,714 €) in April 2021, as a sign of interest in this study. However, at the time of sending the proposal to the Research Ethics Committee of the Hospital Clinic of Barcelona, funding for the whole project is pending.

17. PUBLICATION POLICY

The principal investigator is responsible for publishing the results of this study in medical journals and meetings. Local principal investigators as well as laboratory researchers involved in this project will be co-authors of any resulting publication and presentation. Centers with a large recruitment rate will be granted to add one additional investigator per each 1000 participants included in the study. Other investigators participating in the study will be part of a collective authorship, mentioned as "on behalf of the miRFec study investigators" and including his/her name in the corresponding annex.

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ANNEX 1. Information sheet

PATIENT INFORMATION SHEET

STUDY TITLE: Prospective, multicenter, comparative, parallel study to validate a microRNA- based fecal test for colorectal cancer screening. The miRFec study.

SPONSOR CODE: miRFec001

SPONSOR: *Fundació Clínic per a la Recerca Biomèdica* (Clinic Foundation for Biomedical Research)

PRINCIPAL INVESTIGATOR: Antoni Castells, MD, PhD; Gastroenterology Department, Hospital Clinic of Barcelona. Phone number: + 34 93 227 57 03

CENTRE: Hospital Clinic of Barcelona

INTRODUCTION

We invite you to participate in a research study. The study has been approved by the Research Ethics Committee according to the current legislation (*Ley de Investigación Biomédica 14/2007*).

Before you decide whether or not you wish to participate in this study, it is important for you to understand why the research is being done and what it will involve.

Please take your time to read the following information carefully and ask us if there is anything that is not clear or if you would like more information. You can also discuss it with friends and relatives if you wish.

If you decide to take part of the study, you will be given a copy of this information sheet and your signed consent form.

PARTICIPATION

Participation in this study is voluntary and you can decide whether or not you want to participate in it. Whatever your decision, it will not affect your relationship with the staff caring. If you wish to withdraw from the study once it has started, you can do so at any time without having to give a reason.

GENERAL DESCRIPTION OF THE STUDY

Colorectal cancer is the third most common cancer and the second leading cause of cancer-related death in the world. Although 90% of patients diagnosed at early stages have an overall survival of more than 5 years, this figure decreases to 10% in patients diagnosed at advanced stages with distant metastasis. In that sense, evidence from several studies has shown that CRC screening is effective and cost-effective in general population.

The most extended strategy worldwide in population-based organized colorectal cancer screening programs is fecal immunochemical testing (FIT), which looks for occult blood in the stool, an early sign of cancer. While exams such as colonoscopy, another exam used for colorectal cancer screening, detect both cancer and premalignant lesions –so-called adenomatous polyps or adenomas–, FIT mainly identify cancer because of their low sensitivity for the detection of

advanced adenomas. Moreover, the relatively low specificity results in a high false-positive rate, leading to a significant number of unnecessary colonoscopies.

A different approach to optimize CRC screening is the use of biomarkers other than hemoglobin, such as microRNAs, which can be detected in either blood or feces and they may contribute to overcome the above-mentioned limitations.

We aim to validate the results recently obtained by our research group, which demonstrated that two microRNAs (miR-421 and miR-27a-3p) along with fecal hemoglobin concentration –the miRFec test– identify patients with advanced colorectal neoplasia (that is, cancer or advanced polyps) more accurately than hemoglobin concentration along among FIT-positive individuals.

The main goal of present study is to compare the sensitivity of the miRFec test with respect to FIT for the detection of patients with advanced colorectal neoplasia among individuals participating in a colorectal cancer screening program. We will also compare specificity, detection rate and cost-effectiveness of the miRFec test with respect to FIT, and sensitivity for detection of cancer and advanced polyps separately.

If you accept to participate in the study, our staff will provide you with a fecal sample collection kit along with instructions for sample collection. The sample must be collected at home before starting bowel preparation, more specifically between 24-72 hours before the day that colonoscopy is scheduled.

The sample must be collected before start taking the laxatives prior to the colonoscopy you should undergo. Once the sample is collected, you must keep it on the fridge until you come to the hospital to perform the colonoscopy. The endoscopy staff will be in charge of collecting and storing the sample.

The study population will consist of individuals aged 50 to 75 without family history of colorectal cancer and individuals aged 30 to 80 with such a familial background, all of them referred to colonoscopy-based colorectal cancer screening.

Eligible individuals will undergo two different examinations –FIT and miRFec test– in a unique stool sample collected at home, which results will be compared to those obtained from the colonoscopy you should undergo.

The study will be carried out in two consecutive stages: phase I to calculate the most adequate threshold of the miRFec test, in which 3600 individuals will be included, and phase II to verify this cut-off value in 6700 additional individuals.

BENEFITS AND RISKS DERIVED FROM YOUR PARTICIPATION IN THE STUDY

There is no direct benefit for individuals participating in the study. However, results obtained in this study will allow us to improve diagnostic capacity of current screening methods, and they may benefit individuals like you in the near future.

Participation in this study will not represent any increased risk in addition to complications related to the colonoscopy you should undergo (use of laxative for bowel preparation, adverse reaction to sedation, or bleeding and perforation if any lesion should be resected).

Participation in the study will not represent any additional medical visit or examination.

Analyses carried out in the stool sample you provide us will be done in our laboratory, so there is no risk for pregnant or lactating women.

Your responsibilities will only consist of collecting the stool sample at home, keep it on the fridge, and bring it to the hospital the day your colonoscopy is performed.

ALTERNATIVE TREATMENTS

Your participation in the study does not change the treatment prescribed by your physician, since you should always take laxatives as bowel preparation for colonoscopy.

CONFIDENTIALITY AGREEMENT

The *Fundació Clínic per a la Recerca Biomèdica* (Clinic Foundation for Biomedical Research), with CIF G-59319681, as responsible for your data processing, informs you that the treatment, communication and transferring of personal data of all participants will comply with EU Regulation 2016/679 of the European Parliament and with April 27, 2016 Council, regarding the protection of people concerning the processing and free circulation of personal data, being compulsory since May 25, 2018. The legal basis that justifies the processing of your data is the consent that appears in this act, according to article 9 of EU Regulation 2016/679.

Data for these studies will be collected and identified by a code, so no information to identify the participants will be included. Only study's Principal Investigator and his collaborators with specific permission will be allowed to relate the data collected in the study with your medical record.

Your identity will not be available to any other person except for a medical emergency or legal requirement. The health authorities, the Research Ethics Committee and authorized personnel by the study Promoter may have access to your identified personal information when necessary, to verify data and study procedures, but always maintaining confidentiality according to the current legislation.

Only coded data will be transferred to third parties and other countries, which in no case will contain information that can directly identify the participant (such as name and surname, initials, address, social security number, etc.). If this transfer occurs, it would be only for the study and guaranteeing confidentiality.

If a transfer of encrypted data is made outside the EU, either to entities related to the hospital center where you participate, to service providers or researchers who collaborate with your doctor, your data will be protected by safeguards such as contracts or other established mechanisms established by data protection authorities.

In addition to the rights that the previous legislation already contemplated (access, modification, opposition and cancellation of data, deletion in the new Regulation) you can now also limit the processing of incorrect data, request a copy or that the data provided for the study can be transferred to a third party (portability). To exercise these rights, or if you want to know more about confidentiality, you should contact the Principal Investigator of the study or the Data Protection Officer of the *Fundació Clínic per a la Recerca Biomèdica* through protecciodades@clinic.cat. They also have the right to contact the Data Protection Agency if you are not satisfied.

Data already collected cannot be removed even if you leave the study, to ensure the validity of the research and to come into effect with legal duties and drug authorization requirements. But no new data will be collected if you decide to stop participating.

It is mandatory for the Investigator and the Promoter to keep the data collected for the study at least 25 years after its completion. Subsequently, personal information will only be kept by the

center for your health care and by the Promoter for other scientific research purposes if the patient has given its consent to do so, and if this is allowed by the law and applicable ethical requirements.

ECONOMIC COMPENSATION

Your participation in the study will not represent any economic cost to you. You will not have to pay for the study tests. On the other hand, you will not receive any economic compensation for participating in the study.

COLLECTION AND USE OF BIOLOGICAL SAMPLES

Participation in this study involves obtaining a stool sample.

By signing this document, you agree that the samples will be obtained from the present study, according to biomedical research Law 14/2007 and Royal Decree 1716/2011, which regulates the use of biological samples in research.

Samples will be stored in the laboratory of *Fundació Clínic per a la Recerca Biomèdica* in Barcelona until they are used for this study. Once the study is finished, leftover samples will be destroyed unless you express your consent to store them at the Biobanc of Hospital Clínic-IDIBAPS at the registered collection number C. 0004006, and use them in future research.

A code to identify your sample will be used. Only the study's Principal Investigator and his collaborators will be able to associate the sample with you. The information obtained from using these samples will be treated in the same way as the rest of the data obtained during this study.

Biological samples transfer for this study is free and voluntary. That means that you will have no rights to any possible commercial benefit from the findings that could result from biomedical research.

Any relevant information that could affect your health or your family members will be notified. If necessary, we will use the data on your medical record to be in touch with you. However, your willingness to receive no information will be respected. If you wish, please check the box found on the consent form.

Additionally, the results obtained if genetic analyses are performed will not be communicated to you or your physician, although you can request them by contacting the study's Principal Investigator.

OTHER RELEVANT INFORMATION

Any new information regarding the test used in the study that may affect your willingness to participate, discovered during your participation, will be communicated to you as soon as possible.

If you decide to withdraw your consent, no new data will be added to the database and you can demand the destruction of all identifiable samples previously retained to avoid further analysis.

You should also know that you can be excluded from the study if the Promoter or study Investigators consider it appropriate either for safety reasons, for any adverse event or because they consider that you are not complying with the established procedures. Anyway, you will receive an adequate explanation of the reason for your withdrawal from the study.

By signing the attached consent form, you accept to comply with the study procedures that have been set forth to you.

ANNEX 2. Participant consent form

PATIENT CONSENT FORM

Study title: Prospective, multicenter, comparative, parallel study to validate a microRNA- based fecal test for colorectal cancer screening. The miRFec study.

Protocol code: miRFec001 (version 2; January 28th, 2022)

Me, (*name and surname*) ……

- I have read the information sheet about the study.
- I have had the opportunity to ask questions about the study.
- I have received enough information about the study.
- I have talked to (*investigator's name*) ……
- I comprehend that my participation is voluntary.
- I comprehend that I can withdraw from the study at any time:
    - Whenever
    - Without giving any reason
    - Without my medical care or legal rights being affected.

- According to the provisions of EU Regulation 2016/679 of the European Parliament and of the April 26th, 2016 Council about the protection of natural persons regarding personal data processing and free circulation of data, I declare that I have been informed of the existence of a file or a personal data processing, the purpose of collecting these data and the information receivers.

- I give my consent to participate in this study.

Participant signature
Date: ___/___/_____

Investigator signature
Date: ___/___/_____

Wish to be informed of any relevant information obtained from the study that might be important for my health:

☐ YES ☐ NO

I give my consent to store and keep remaining samples, so they can be used in future research:

☐ YES ☐ NO

Participant signature
Date: ___/___/_____

Investigator signature
Date: ___/___/_____

ANNEX 3. Instructions for fecal sample collection

English

OC-Auto Sampling Bottle 3

(For OC-SENSOR series)

REF V-PZ25

REF V-PZ26

INTENDED USE

The OC-Auto Sampling Bottle 3 is designed as a specimen container for measurement of haemoglobin in faeces in using together with automated faecal occult blood analysers. Therefore it is ideally suited for use as a sample collection device that can be used to easily collect a constant amount of faeces.

PROCEDURE FOR SPECIMEN COLLECTION

1. Remove the green cap by turning to the left and pulling upwards.
2. Collect the faecal sample with the sampling probe by scraping from different areas of the surface of the faeces.
   Collect the amount enough to cover the groove of the probe.
3. Insert the sampling probe to the sample collection device and tighten the cap. Do not repeat more than once.
4. Shake the bottle vigorously up and down several times.

Note.
If the faeces is hard, moisten it with water before collecting the sample using the sampling probe.

STORAGE

The sample collection device is stable until the date printed on the label assuming the bottle remains unopened at a storage temperature of 1-30°C.

STABILITY AFTER SAMPLING

Performance testing with the sample collection device demonstrated that samples stored at 2-10°C for 28 days have 95±14.7%, at 25°C for 7 days have 96±20.4%, for 14 days have 93±23.5%, and at 30°C for 7 days have 89±20.5%, for 14 days have 84±23.6% of haemoglobin recovery (in-house data, recovery rate shown as mean±2SD). However, the haemoglobin in some samples may undergo rapid denaturation or degradation, resulting in false negatives. Therefore, samples should be stored at 2-10°C and analysed as soon as possible.

COMPOSITION AND DESCRIPTION

Sampling bottles contain buffer (HEPES; N-2-hydroxyethylpiperazine-N'-2-ethane- sulfonic acid, 2mL). Sampling bottles are made of polypropylene (PP) and their bottoms sealed with two layers of aluminium. Sampling probes are made of acrylonitrile butadiene styrene (ABS) resin, and filters and collection bags are made of polyethylene (PE).

WARNINGS AND PRECAUTIONS

1. For *in vitro* diagnostic use only.
2. Do not pour out any fluid in the sample collection device, or add water to it.
3. Do not break the aluminum seal.
4. Do not obtain faecal samples during menstruation.
5. Do not use the sample collection device by placing it in direct contact with the body.
6. Do not use the sample collection device for any purpose other than collecting faecal samples.
7. Store the sample collection device in a location that is out of the reach of children.
8. Perform the measurement as soon as possible after sample has been transported. If analysis is not immediately possible, store the sample collection device in the refrigerator at 2-10°C and analyse as soon as possible.
9. After removing the sample from the refrigerator, be sure to completely return it to the room temperature before using.
10. Do not use the sample collection device that has passed its expiration date.
11. The test sample may contain microorganisms. Therefore use caution when handling. After use, all samples and other materials must be considered to carry a risk of infection and must be treated accordingly.
12. Dispose of used reagents and containers as medical waste in accordance with local regulations.
13. The buffer in the sampling bottles contains a toxic material, sodium azide (NaN_3 <0.1%). Be sure not to contact with skin, eyes, and mouth. In case of exposure, wash vigorously with water and call a doctor for treatment advice.

PRODUCT CODE, PRODUCT NAME AND STORAGE

Product code	Product name	Contents	Storage
V-PZ25	OC-Auto Sampling Bottle 3	100 bottles	1-30°C
V-PZ26	OC-Auto Sampling Bottle 3 without barcode	100 bottles	1-30°C

REFERENCE

1. T. Takeshita et al.: Journal of Coloproctology, 38: 780-783, 1985.

ANNEX 4. Staging according to American Joint Committee on Cancer (8th version)[17]

Primary tumor (T)

- Tx: primary tumor cannot be assessed
- T0: no evidence of primary tumor
- Tis: carcinoma in situ, intramucosal carcinoma (involvement of *lamina propria* with no extension through *muscularis mucosae*)
- T1: tumor invades submucosa (through the *muscularis mucosae* but not into the *muscularis propria*)
- T2: tumor invades *muscularis propria*
- T3: tumor invades through the *muscularis propria* into the pericolorectal tissues
- T4a: tumor invades through the visceral peritoneum (including gross perforation of the bowel through tumor and continuous invasion of tumor through areas of inflammation to the surface of the visceral peritoneum)
- T4b: tumor directly invades or adheres to other adjacent organs or structures

Regional lymph nodes (N)

- Nx: regional lymph nodes cannot be assessed
- N0: no regional lymph node metastasis
- N1: metastasis in 1-3 regional lymph nodes
  - N1a: metastasis in 1 regional lymph node
  - N1b: metastasis in 2-3 regional lymph nodes
  - N1c: no regional lymph nodes are positive but there are tumor deposits in the subserosa, mesentery or nonperitonealized pericolic or perirectal / mesorectal tissues
- N2: metastasis in 4 or more regional lymph nodes:
  - N2a: metastasis in 4-6 regional lymph nodes
  - N2b: metastasis in 7 or more regional lymph nodes

Distant metastasis (M)

- M0: no evidence of tumor in other sites or organs
- M1: distant metastasis:
  - M1a: metastasis confined to 1 organ or site without peritoneal metastasis
  - M1b: metastasis to 2 or more sites or organs is identified without peritoneal metastasis
  - M1c: metastasis to the peritoneal surface is identified alone or with other site or organ metastasis

Stage I	T1	N0	M0
	T2	N0	M0
Stage IIa	T3	N0	M0
Stage IIb	T4a	N0	M0
Stage IIc	T4b	N0	M0
Stage IIIa	T1	N1	M0
	T1	N1c	M0
	T1	N2a	M0
	T2	N1	M0
	T2	N1c	M0
Stage IIIb	T1	N2	M0
	T2	N2a	M0
	T2	N2b	M0
	T3	N1	M0
	T3	N1c	M0
	T3	N2a	M0
	T4	N1	M0
	T4	N1c	M0
Stage IIIc	T3	N2b	M0
	T4a	N2a	M0
	T4a	N2b	M0
	T4b	N1	M0
	T4b	N2	M0
Stage IV	Tx	Nx	M1